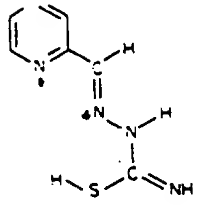

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state the geometric isomers of these thiosemicarbazones exist as thione tautomers.

Experimental Section

Melting points were determined on a Kofler micro hot bench and are uncorrected. NMR spectra were obtained with a JEOL JNM C60-HL spectrometer [sodium 3-(trimethylsilyl)propanesulfonate as internal standard]. The IR spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer. TLC was carried out on TLC plates prepared with silica gel GF_{254} Merck (EtOAc as eluent). Spots were detected with an UV lamp (λ 254 nm). For column chromatography silica gel 60 Merck was used.

(*E*)- and (*Z*)-2-Formylpyridine and 1-Formylisoquinoline Thiosemicarbazones [(*E*)- and (*Z*)-PT, (*E*)- and (*Z*)-IQ-1]. The geometric isomers were prepared by heating for 45 min the corresponding aldehydes (0.03 mol) in 100 ml of EtOH with an equimolecular amount of thiosemicarbazide. The products, which separated as pale yellow crystals by cooling the reaction mixture, consist of *E* isomers contaminated by a small amount of *Z* isomers (as was checked by NMR and TLC); they were collected by filtration and purified by crystallization from EtOH: yields 75% for (*E*)-PT and 70% for (*E*)-IQ-1. The evaporation of the filtrate gave a residue which was dissolved with a mixture of MeOH–EtOAc and chromatographed on a silica gel column eluting with EtOAc. Removal of the solvent from the portion of the eluate containing the faster eluted isomers gave (*Z*)-PT and (*Z*)-IQ-1: yields 3 and 5%, respectively.

***Z* Isomers by Isomerization of *E* Isomers.** A suspension of 1 g of *E* isomer and 5 g of silica gel (0.08 mm) Merck in 50 ml of MeOH was heated for 60 min. Evaporation of the solvent gave a residue which was extracted with an hot mixture of MeOH–EtOAc. The solution was chromatographed on a silica gel column with EtOAc as eluent. Removal of the solvent from the portion of the eluate containing the faster eluted isomers gave (*Z*)-PT and (*Z*)-IQ-1: yields 25 and 30%, respectively.

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Synthesis and Antihypertensive Activity of 1-Amino-3,4-dihydroisoquinolines

Guy D. Diana,* W. Banks Hinshaw, and Harlan E. Lape

Sterling-Winthrop Research Institute, Rensselaer, New York 12144. *Received February 25, 1976*

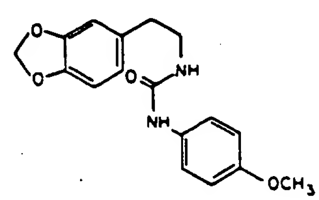
A series of 1-substituted 3,4-dihydroisoquinolines has been synthesized and screened for antihypertensive activity in the renal hypertensive rat. The 1-hydrazino homologue 6 and the corresponding acetaldehyde 25 and acetone 23 hydrazones exhibited good activity but were less effective than hydralazine (7).

The wide variety of pharmacological activity associated with amidines has been well documented[1] and includes such diverse types as antibacterial, hypoglycemic, and antihypertensive activities. 1-Amino-3,4-dihydroisoquinolines (3) represent a class of cyclic amidines, some of which have been reported to exhibit cardiovascular and pressor activity[2] as well as antitussive and antifibrillatory activity.[3] We wish to describe the synthesis and antihypertensive activity of several homologues in this series.

Chemistry. The synthetic sequence chosen to obtain the dihydroisoquinolines is outlined in Scheme I. This procedure was not applicable to the aromatic homologues (R_2 or R_3 = aryl).

The imino esters 2, obtained from 1[4] via Meerwein reagent,[5] were condensed with the appropriate amine producing the cyclic amidines[6] 3 which were isolated in most cases as their sulfate salt.

Aromatic amines did not react with the imino esters 2, even under extreme conditions. The *N*-arylamidines 3a (R_2 = H; R_3 = Ar) could be prepared in fair yield by cyclization of the appropriate urea. For example, 4 provided amidine 5 when treated with a mixture of phosphorus oxychloride and phosphorus pentoxide.



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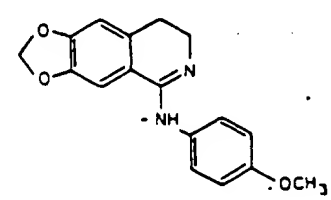

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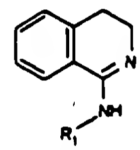
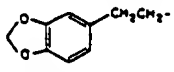
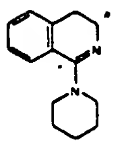
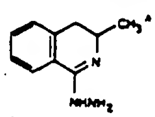
The hydrazino homologue 6 was synthesized because of its similarity to hydralazine 7, a useful antihypertensive

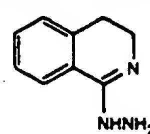
Table I



No.	R_1	Mp, °C (uncor)	Yield, % (from imin ester)	Formula	Analyses[a]	AED[b]
6	H_2N-	55–57	82/85[c,d]	$C_9H_{11}N_3$	C, H, N	30
3b	(R_2 = H; R_3 = NH_2)	147–148	91	$C_9H_{11}N_3O\cdot HBF_4$	C, H, N	>50
9	H-[d]	133–134	72	$C_9H_{10}N_2\cdot HCl$	C, H, N	>50
10	*n*-C_6H_{13}-	147.5–149.5	74	$C_{15}H_{22}N_2\cdot H_2SO_4$	C, H, N	>50
11	$(CH_3)_2NCH_2CH_2$-	270–271	90[e]	$C_{13}H_{19}N_3\cdot 2HCl$	C, H, N	>50
12	C_5H_4N-4-CH_2-	230–230.5	92[e]	$C_{15}H_{15}N_3\cdot H_2SO_4$	C, H, N	>50
13	3,4-H_3CO-C_6H_3-CH_2CH_2-	219–221	73[e]	$C_{19}H_{22}N_2O_2\cdot H_2SO_4$	C, H, N	>50
14		161–162.5	76[e]	$C_{18}H_{18}N_2O_2\cdot H_2SO_4$	C, H, N	>50
15	3-Cl-4-H_2N-C_6H_3-CH_2CH_2-	210–211	52[e]	$C_{17}H_{18}ClN_3\cdot 1.5H_2SO_4$	C, H, N	>50
16	3,4,5-$H_3COC_6H_2$-CH_2CH_2-	195–207	38[e]	$C_{20}H_{24}N_2O_3\cdot H_2SO_4$	C, H, N	>50
17	3,4-OH-C_6H_3-CH_2CH_2-	212–213	98[c]	$C_{17}H_{18}N_2O_2\cdot HCl$	H, N; C[k]	>50
18	4-$(C_2H_5)_2NCH_2CH_2O$-C_6H_4-CH_2-	293–294	36[e]	$C_{22}H_{29}N_3O\cdot C_{10}H_8O_6S_2\cdot 3H_2O$[f]	C, H, N	>50
19	4-CH_3O-C_6H_4-	105–106.5	41[g]	$C_{16}H_{16}N_2O$	C, H, N	>50
20		230–232	88[e]	$C_{14}H_{18}N_2\cdot HCl$	C, H, N	>50
21	$C_6H_5CH_2O$-	235–237	76[c]	$(C_{16}H_{16}N_2O)_2\cdot C_{10}H_8O_6S_2$[f]	C, H, N	>50
22	$(CH_3)_2NH$-	237–240	69[i]	$C_{11}H_{15}N_3\cdot HCl$	C, H, N	>50
23	$(CH_3)_2C{=}N$-	184–185.5	85[j]	$C_{12}H_{15}N_3\cdot H_2SO_4$	C, H, N	15
24	$CH_3(CH_2)_3CH{=}N$-	147–148	90[j]	$C_{14}H_{19}N_3\cdot HCl$	C, H, N	>50
25	$CH_3CH{=}N$-	152–154	62[j]	$C_{11}H_{13}N_3\cdot H_2SO_4$	C, H, N	15
26	c-$C_6H_{10}{=}N$-	139–141	86[j]	$C_{15}H_{19}N_3\cdot H_2SO_4$	C, H, N	>50
27	$CH_3CH{=}C(CH_3)CH{=}N$-	231–232	80[j]	$C_{14}H_{17}N_3\cdot H_2SO_4$	C, H, N	>50
28	$C_6H_5CH{=}N$-	226–228	93[j]	$C_{16}H_{15}N_3\cdot HCl$	C, H, N	>50
29	2,3-H_3CO-C_6H_3-$CH{=}N$-	222–223	74[j]	$C_{18}H_{19}N_3O_2\cdot HCl$	C, H, N	>50
30	4-OH-3,5-H_3CO-C_6H_2-$CH{=}N$-	232–233	81[j]	$C_{18}H_{19}N_3O_3\cdot HCl$	C, H, N	>5
31		174–176	75	$C_{10}H_{13}N_3\cdot HCl$	C, H, N	40
7	Hydralazine					10

[a] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [b] Average effective dose in mg/kg. See text. [c] Prepared from the free base in refluxing ethanol; see Experimental Section. [d] See ref 3, HI salt. [e] Prepared from free base without solvent; see Experimental Section. [f] 1,5-Naphthalenedisulfonate salt. [g] Prepared from $C_6H_5CHCH_2NHC(=O)NH$-C_6H_4-OCH_3 in the same manner as compound 8. [h] Represents complete structural formula. [i] Prepared from hydrochloride in refluxing ethanol; see Experimental Section. [j] Yield based on conversion of corresponding hydrazine to hydrazone. [k] C: calcd, 64.05; found, 63.63.

agent. Treatment of **2a** with 98% hydrazine produced



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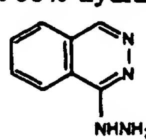

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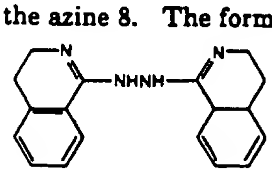
in addition to **6** the azine **8**. The formation of this side



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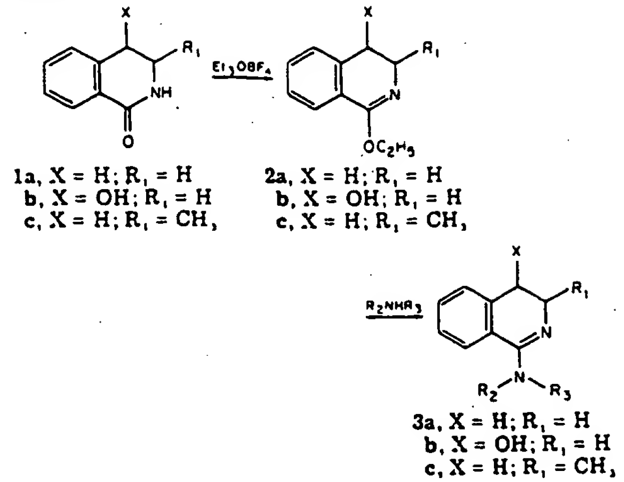
product was readily avoided by utilizing hydrazine monohydrochloride in this reaction yielding **6** as its monohydrochloride.

The 4-hydroxy-substituted imino ester **2b** was available from 3,4-dihydro-4-hydroxy-1(2*H*)-isoquinolinone[7] **1b** by careful treatment with Meerwein reagent. Conversion f the tetrafluoroborate salt of **2b** to **3b** (R_2 = H; R_3 = NH_2) with 98% hydrazine proceeded as in the foregoing example. Table I summarizes the compounds prepared in this series.

Pharmacology. The compounds were evaluated in renal hypertensive rats (bilateral encapsulation method).[8] Rats were rendered 70–80% hypertensive after 6–8 weeks and were considered hypertensive when their blood pressure reached a level of 160 mm or greater. The compounds were administered orally, one dose, to three rats, and blood pressures were measured at 2, 6, and 24 h postmedication. Measurements were made with a photoelectric tensometer.[9] Results were recorded as AED_{50} which was that dose producing a lowering of blood pressure in 50% of the animals, to a level of 130 mm or less. Those compounds which exhibited an AED_{50} of 50 mg/kg were then administered at dose levels of 30 and 15 mg/' ɔ three rats each. Hydralazine (**7**) was used as a stan. i to compare the antihypertensive effects. The hydrazino homologue **6** showed an AED_{50} of 30 whereas the 3-methyl

Scheme I



1a, X = H; R_1 = H
b, X = OH; R_1 = H
c, X = H; R_1 = CH_3

2a, X = H; R_1 = H
b, X = OH; R_1 = H
c, X = H; R_1 = CH_3

3a, X = H; R_1 = H
b, X = OH; R_1 = H
c, X = H; R_1 = CH_3

homologue 21 was slightly less active and the 4-hydroxy homologue **3b** (R_2 = H; R_3 = NH_2) was inactive. The acetone and acetaldehyde hydrazones **23** and **25**, respectively, were twice as active as the parent compound **6** but were less effective than hydralazine. None of the other compounds prepared exhibited any antihypertensive activity.

Discussion

It becomes quite obvious from the results in Table I that the presence of the hydrazine group is necessary for antihypertensive activity. In view of the activity of hydralazine, the increase in activity produced by the acetaldehyde and acetone hydrazones of **6** was quite surprising. Whether, in the form of its hydrazones, compound **6** is carried more effectively to the site of action and then liberated as the free hydrazine is at this point uncertain.

Experimental Section

1-Ethoxy-3,4-dihydroisoquinoline (2a). To 113.6 g (0.8 mol) of freshly distilled boron trifluoride etherate dissolved in 50 ml of absolute Et_2O was added dropwise over 1 h 37.0 g (0.4 mol) of epichlorohydrin. After 2.5 h of stirring, the supernatant liquid was decanted and the waxy solid washed four times by decantation with Et_2O. This solid was then dissolved in 100 ml of CH_2Cl_2 and a solution of 30.8 g (0.21 mol) of 3,4-dihydro-1-isoquinolinone[4] added at a rate sufficient to maintain reflux. The mixture was stirred at room temperature for 18 h, then cooled in an ice bath, and mixed with 100 ml of 50% K_2CO_3 and 75 ml of H_2O. The organic layer was separated, dried over $MgSO_4$, and concentrated to provide 36 g of liquid which was distilled at 10 mm and 111–112 °C to provide 33.5 g of **2a**, 91%. Anal. ($C_{11}H_{13}NO$) C, H, N.

1-Ethoxy-3-methyl-3,4-dihydroisoquinoline (2c). The preparation was similar to that above providing a colorless liquid, bp 128–129 °C (15 mm). Anal. ($C_{12}H_{15}NO$) C, H, N.

1-Ethoxy-3,4-dihydro-4-isoquinolol Tetrafluoroborate (2b). A solution of triethyloxonium fluoroborate (0.147 mol) in 200 ml of CH_2Cl_2 was prepared as above and added dropwise to a solution of 3,4-dihydro-4-hydroxy-1(2*H*)-isoquinolinone[5] (**1b**), 21.75 g (0.133 mol), in 200 ml of CH_2Cl_2. After 3 h, the mixture was filtered and concentrated in vacuo. The white solid (21 g) so obtained was recrystallized from EtOH–Et_2O to provide 15.4 g (55%) of 1-ethoxy-3,4-dihydro-4-isoquinolinol tetrafluoroborate **2b**, mp 121–123 °C. Anal. ($C_{11}H_{13}NO_2 \cdot HBF_4$) C, H, N.

1-(Benzyloxyamino)-3,4-dihydroisoquinoline 1,5-Naphthalenedisulfonate (21). A mixture of 10 g (0.57 mol) of **2a** (R_1 = H), 9.15 g (0.57 mol) of benzyloxyamine hydrochloride, 12 g (0.57 mol) of Na_2CO_3, and 50 ml of absolute alcohol was stirred at 25 °C for 7 h. The solid was removed by filtration and the filtrate concentrated to dryness. The residual oil was extracted with ether and the ethereal solution washed with water and dried. Removal of the solvent gave 14.4 g of colorless oil which was taken up in glacial HOAc (50 ml), diluted with 0.5 vol of H_2O, and treated with an aqueous solution of 9.5 g (0.236 mol) of disodium naphthalene-1,5-disulfonate in 50 ml of H_2O. A solid (24 g) separated and was recrystallized from MeOH–H_2O. **21** (16.2 g, 79%) was obtained, mp 235–237 °C. Anal. [$2(C_{16}H_{16}N_2O) \cdot C_{10}H_8O_6S$] C, H, N.

1-Piperidino-3,4-dihydroisoquinoline (20). A solution of 11 g (0.0629 mol) of **2a** in 27 g (0.316 mol) of piperidine was refluxed under a stream of nitrogen for 48 h, after which time the excess piperidine was removed in vacuo. The residual oil was distilled: bp 91–97 °C (0.01 mm); yield 11.9 g (88.3%). The base was converted to its HCl salt by the addition of excess ethereal HCl to an ethereal solution of the base. The resulting white solid was recrystallized from EtOH–$(C_2H_5)_2O$: 14.1 g; mp 230–232 °C. Anal. ($C_{14}H_{18}N_2 \cdot HCl$) C, H, N.

1-Hydrazino-3,4-dihydroisoquinoline (6). A solution of 2.0 g (0.011 mol) of 1-ethoxy-3,4-dihydroisoquinoline (**2a**) in 10 ml of absolute EtOH was added to a solution of 1.83 g (0.057 mol) of 95% hydrazine in 5 ml of EtOH and heated at reflux. After 0.5 h, the mixture was cooled, the EtOH removed in vacuo, and the residue dissolved in CH_2Cl_2 and washed three times with H_2O. The CH_2Cl_2 solution was dried over Na_2SO_4, filtered, and concentrated and the residue crystallized from Et_2O–pentane to provide 1.5 g (82%) of **6**, mp 55–57 °C. Anal. ($C_9H_{11}N_3$) C, H, N.

Cyclohexanone 3,4-Dihydro-1-isoquinolylhydrazone Sulfate (26). A solution of 9.4 g (0.058 mol) of **6** and 6.3 g (0.064 mol) of cyclohexanone in 300 ml of ether was allowed to stand at 25 °C for 2 h and then treated with an excess of 10% ethereal H_2SO_4. The oily precipitate was dissolved in hot isopropyl alcohol, treated with activated carbon, and recrystallized from $CH_3CHOHCH_3$–Et_2O to provide 17.3 g of **26**, mp 139–141 °C. Anal. ($C_{15}H_{19}N_3 \cdot H_2SO_4$) C, H, N.

3,4-Dihydro-1(2*H*)-isoquinoline Azine (8). To a solution of 3.9 g (0.024 mol) of 1-hydrazino-3,4-dihydroisoquinoline (**6**) in 20 ml of MeOH at 25 °C was added dropwise a solution of 5.25 g (0.03 mol) of 1-ethoxy-3,4-dihydroisoquinoline (**2a**) in 20 ml of MeOH. After the addition was complete, the mixture was refluxed for 12 h, cooled, and filtered. The yellow solid obtained by evaporation of the filtrate was recrystallized from $CH_3CHOHCH_3$ to provide 2.8 g (40%), mp 178–179 °C. Anal. ($C_{18}H_{18}N_4$) C, H, N.

1-(4-Methoxyphenyl)-3-(3,4-methylenedioxyphenethyl)urea (4). To a mechanically stirred solution of 15.0 g (0.093 mol) of 3,4-methylenedioxyphenethylamine in 100 ml of C_6H_6 was added dropwise a solution of 13.8 g (0.093 mol) of *p*-methoxyphenyl isocyanate in 50 ml of C_6H_6. After 2 h, the mixture was stirred with 100 ml of pentane and filtered, the white solid being washed several times with pentane. This provided 28 g of the urea (97%) which could be recrystallized from $CH_3CHOHCH_3$, mp 177–179 °C. Anal. ($C_{17}H_{18}N_2O_4$) C, H, N.

1-(*p*-Anisidino)-3,4-dihydro-6,7-(methylenedioxy)isoquinoline Hydrochloride Hemiethanolate (5). To a solution of 16.5 g (0.053 mol) of **4** in 75 ml of warm $POCl_3$ was added 20 g of P_2O_5. The solution was stirred on a steam bath for 30 min, then poured on ice, and made strongly basic with 35% NaOH. Filtration provided 20 g of a yellow solid which was washed with cold H_2O. Dissolution of this solid in MeOH followed by acidification with saturated ethereal HCl and precipitation with Et_2O provided a yellow solid which could be recrystallized from EtOH–Et_2O to give 12.4 g (66%) of the expected salt, mp 255–256 °C. Anal. ($C_{17}H_{16}N_2O_3 \cdot HCl \cdot 0.5C_2H_5OH$) C, H, N.

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Synthesis and Biological Activity of Potential Antimetabolites

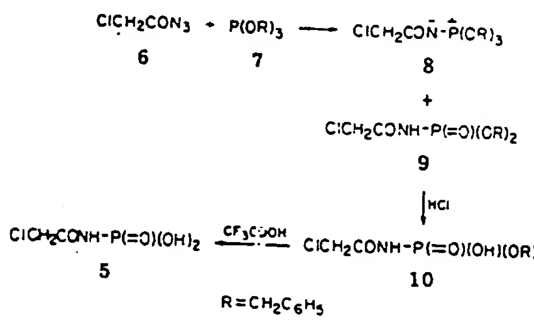
G. I. Glover,* S. O. Nelson, and G. R. Kaeder

Department of Chemistry, Texas A&M University, College Station, Texas 77840. *Received May 10, 1976*

Several known α-amino acid analogues and a new compound, *N*-chloroacetylphosphoramidate, a carbamyl phosphate analogue, were screened as antitumor agents. All gave 50% growth inhibition of cultures of human epidemeroid carcinoma of the nasopharynx at dosage levels of 2–8 μg/ml while showing no activity against L1210 lymphoid leukemia in vivo in BDFi mice.

In view of the potential of compounds containing the alkylating functional group, $-COCH_2Cl$, as antimetabolites, *N*-chloroacetylphosphoramidate (5) was prepared as an analogue of carbamyl phosphate, and it and α-aminochloromethyl ketones (compounds 1–4) containing this functional group in lieu of the carboxyl of tyrosine,[1] phenylalanine,[2] leucine,[3] and lysine[4] were screened as antitumor agents.

Chemistry. The chloromethyl ketones 1–4 were prepared as referenced above. The monobenzylcarbamyl phosphate analogue 10 was prepared by modification of the reported method for synthesis of diethyl *N*-chloroacetylphosphoramidate.[5] In the synthesis of the diethyl ester of 5, it was necessary to treat the intermediate triethoxyphosphonium salt (8 with R = ethyl) with hydrogen chloride to remove one of the ethyl groups. The crude



product from the reaction of 6 with 7 was a mixture of 8, 9, and benzyl chloride rather than 8 alone. After treatment of the mixture with hydrogen chloride gas we isolated 10, which is the monobenzyl ester of 5. This ester was crystalline and all spectral and analytical data are in agreement with the structure proposed. Since the first benzyl group was so labile, we treated 10 with trifluoroacetic acid under mild conditions at 25 °C giving complete removal of the blocking group. The product was isolated as the dilithium salt which had IR and NMR absorptions consistent with the structure even though the analysis for carbon was not quite acceptable.

Biological Results and Discussion. Compounds 1–5 were submitted to Drug Research and Development, National Cancer Institute, and were screened in their routine preliminary program in vitro in cell culture using human epidemeroid carcinoma of the nasopharynx (90 KB system) and in vivo using BDFi mice as the host for L1210 lymphoid leukemia (2 LE system).[6] No activity was noted in the latter assay while all the compounds showed growth inhibition in the in vitro assay at concentrations in the 2–8 μg/ml range. Compounds 1, 2, 4, and 5 are sufficiently

Table I. Antitumor Activity of Potential Antimetabolites[a]

Compd	Structure[b]	ED_{50}, μg/ml[c]
1	R = p-OH-C_6H_4	2.1
2	R = C_6H_5	4
3	R = $(CH_3)_2CH$	7.6
4	R = $H_2N(CH_2)_4$	2.7
5	$Li_2(O_3PNHCOCH_2Cl)$	2.5

[a] Compounds were assayed in cell cultures of human epidemeroid carcinoma of the nasopharynx (90 KB system).[6] [b] Compounds 1-4 have the general structure $RCH_2CH(NH_2)COCH_2Cl$, administered as the hydrochloride salts. [c] ED_{50} is the concentration giving 50% growth inhibition compared to untreated controls.

active to warrant secondary screening. These systems are routinely employed by the NCI for synthetics and extensive testing has been limited by the present availability of the compounds.

The amino acid analogues 1–4 could function by inhibiting a variety of enzymes that bind the corresponding amino acids as substrates, but irreversible inhibition of transport of essential amino acids in malignant cells could also lead to growth inhibition. The latter mode of action is suggested by other work. Phenylalanine-deficient diets in rats lead to decreased tumorigenesis and to decreased growth rates of already formed tumors.[7] Treatment of murine leukemic (L5178Y) lymphoblasts with phenylalanine ammonia lyase inhibits the growth of these cells also by depriving them of phenylalanine.[8] Growth inhibition of SV40-transformed 3T3 cells by dibutyryl cAMP is accompanied by decreased capability for transport of leucine.[9]

The analogue 5 may be functioning as an antagonist of carbamyl phosphate. The reaction between 5 mM aspartate and 4 mM carbamyl phosphate catalyzed by 0.1 μg/ml of aspartate transcarbamylase from *Escherichia coli* is 60% inhibited by 66 mM 5 while the same concentration of phosphate (a weak competitive inhibitor) gave only 24% inhibition. No irreversible inhibition of this enzyme by 5 was noted even on prolonged incubations in the presence and absence of aspartate, but this does not preclude the possibility of irreversible inhibition by 5 of mammalian enzymes that bind carbamyl phosphate.

Experimental Section

NMR spectra were recorded on a Varian T-60 spectrometer using tetramethylsilane as an internal reference. Infrared spectra were obtained using a Perkin-Elmer 237B grating spectrophotometer. Melting points were obtained in capillary tubes and are reported uncorrected. Elemental analyses were within 0.4% of theoretical values except where noted and were performed by Galbraith Laboratories. *Note* that hydrazoic acid is toxic and